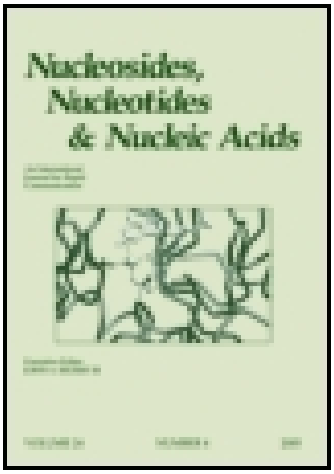

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Acyclic Nucleotide Analogues and Related Compounds

Antonín Holý [a] & Hana Dvořáková [a]
[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic , 166 10, Praha, 6, (The Czech Republic)
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ACYCLIC NUCLEOTIDE ANALOGUES AND RELATED COMPOUNDS

Antonín Holý * and Hana Dvořáková
Institute of Organic Chemistry and Biochemistry,
Academy of Sciences of the Czech Republic, 166 10 Praha 6 (The Czech Republic)

Abstract. Acyclic nucleotide analogues bearing amino- and N-substituted amino groups in the side chain were prepared by alkylation of the bases with corresponding oxiranes and subsequent introduction of phosphonomethyl ether function. Novel enantiomeric synthons for the preparation of HPMP-compounds were prepared from a common intermediate and applied to syntheses of novel compounds (e.g. 8-azaguanine derivatives).

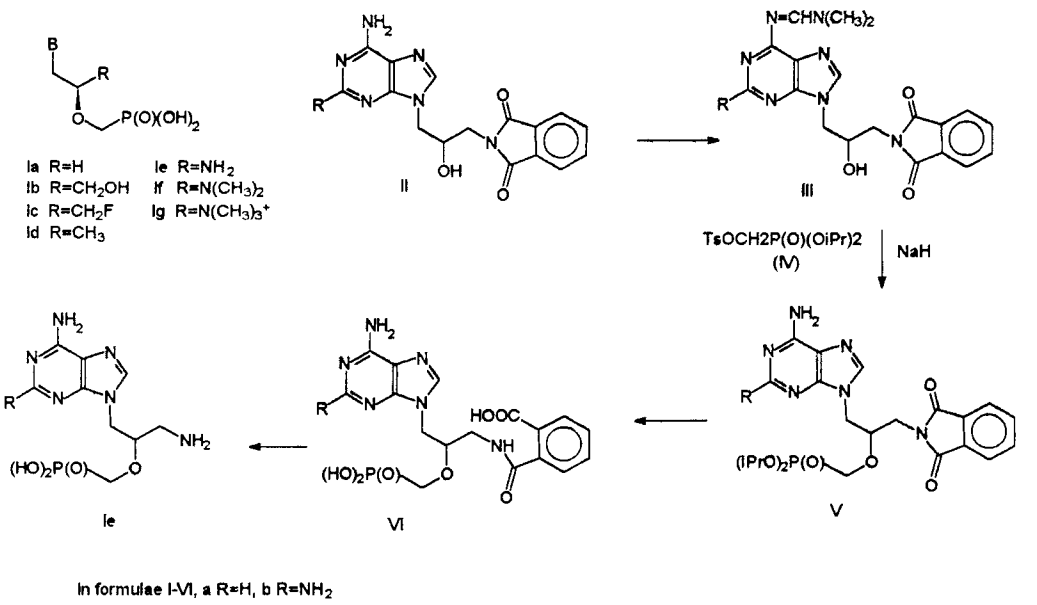
INTRODUCTION

N-Phosphonomethoxyalkyl derivatives of pyrimidine and purine bases (I) possess antiviral and cytostatic activities of considerable interest[1,2]. In our preceding SAR studies we have been able to show that the biological activity *per se* reflects the nature of the heterocyclic base while the specificity of the antiviral action is determined largely by the structure of the side chain. Our studies also show that the structural margins of the modification of both the heterocyclic system and the side chain are narrow. The choice of the base is restricted to adenine, 2-aminopurine, 2,6-diaminopurine and guanine and some of their N^6-substituted derivatives (the antiviral activity of cytosine derivative HPMPC is a rare exception); it determines solely the magnitude of the biological effect. Later on, we have reported antiviral activity of some 1-deaza-[3] and 3-deazapurine congeners[3,4] and, finally, of certain 2-aza-[5] and 8-azaadenine derivatives[5,6];however, our data obtained with isomeric N-(2-phosphonomethoxyethyl)-8-azaguanines[6] contradicted to some extent the observations recently published by Italian authors[7]. Therefore, we have examined the synthesis and activity of the whole group of acyclic phosphonate analogues derived from 8-azaguanine.

It has been concluded that the structure of the side chain linked to the base estimates the character of the antiviral effect. While the HPMP-derivatives (Ib) act against DNA viruses, the effect of FPMP (Ic)[8] and PMP (Id) compounds[9] is selectively anti-retroviral. PME derivatives (Ia) lacking a substituent at the β-position altogether act both against DNA and retroviruses[1]. The cytostatic activity is limited to PME and/or PMP-derivatives only[1,2]. On the other hand, of the group tested, only HPMP-derivatives are active against Plasmodium sp.[10]. The influence of the β-substituent on biological activity in these compounds is a subject of our continuing investigation. We have recently reported[11] that, except for the methyl derivatives (Id), none of the alkyl, cycloalkyl or aralkyl derivatives of the type I had any antiviral activity. The hydroxyl group as a part of the hydroxymethyl substituent seems to determine the specificity. In the adenine derivative HPMPA, its etherification, esterification or replacement by amino group (Ie) quenches entirely the antiviral activity[12,13]. However, certain activity is reportedly preserved in the guanine counterpart of the latter compound[14]. We have observed earlier that such effects of guanine derivatives are often caused by the extreme toxicity of guanine derivatives that is most probably due to disturbance of purine nucleotide pool resulting from strong purine nucleoside phosphorylase inhibition[15]. Therefore, we have studied in detail the synthesis and properties of diverse aminoalkyl derivatives of acyclic nucleotide phosphonates bearing N-substituted aminoalkyl groups at the β-position.

RESULTS

Synthesis of 3-amino-2-phosphonomethoxypropyl derivatives. Our original method[13] for the synthesis of the aminoalkyl derivative Ie (A=adenin-9-yl) made use of N-(3-azido-2-hydroxypropyl)adenine that was transformed into the phosphonate and converted to the final amino compound by hydrogenation. Since the latter reaction is not compatible with some heterocyclic systems (e.g. cytosine) we needed to elaborate an alternative procedure avoiding hydrogenation. Such an alternative served for the preparation of racemic compounds Ie (Scheme 1). The starting phtalimido derivatives II can be easily obtained by alkylation of the base with phtalimidomethyloxirane in the presence of cesium carbonate[16]. On treatment with dimethylformamide dimethylacetal, the amino



Scheme 1

function of the heterocyclic base can be protected by amidine formation without affecting the phtalimido group; the resulting product III is subjected to condensation with the key synthon IV[17] in the presence of excess NaH. The resulting phtalimido-diester V (or VI) loses simultaneously the ester and phtalimide (phtalamoyl) groups on treatment with bromotrimethylsilane (BTMS) thus affording the free phosphonate Ie. This route was applied to the synthesis of adenine, 2,6-diaminopurine, guanine and cytosine derivatives of the type Ie.

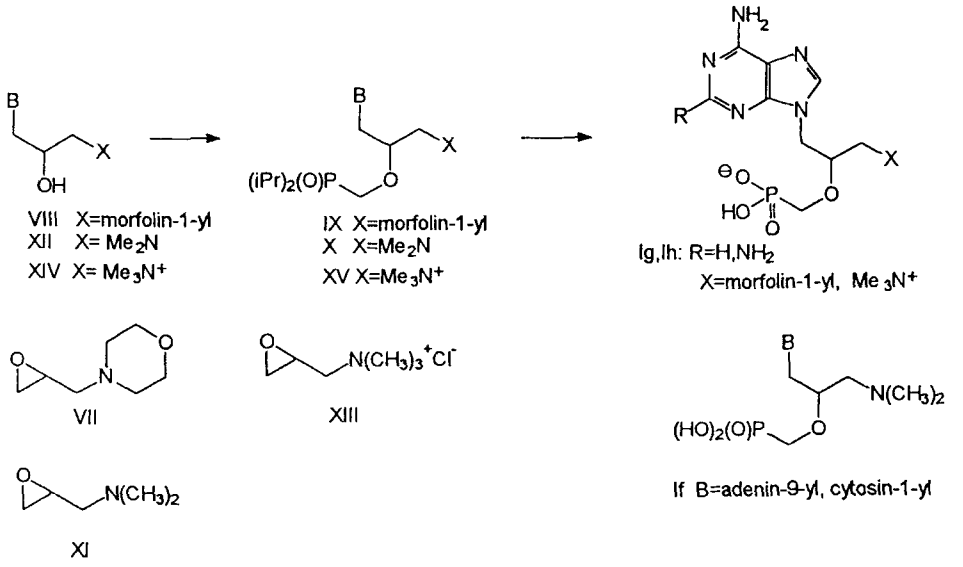
Synthesis of N-substituted 3-amino-2-phosphonomethoxypropyl derivatives. The same strategy was applied to the synthesis of N,N-substituted 3-aminomethyl derivatives (Ig). Thus, alkylation of purine or pyrimidine base with morpholinomethyloxirane (VII) gave the expected 3-morpholino-2-hydroxypropyl derivative VIII. The alkylation produced predominantly the N^9-isomers in the purine series and the N^1-isomer with cytosine. The amidine protection, which was performed by treatment with dimethylformamide dimethylacetal, took place without any side reaction. The introduction of phosphono-

methyl ether group at the 2-hydroxyl function of the intermediates IX proceeded smoothly by condensation with the synthon IV to give diesters X. They were converted to the final products I by reaction with BTMS followed by hydrolysis (Scheme 2).

The starting oxiranes bearing tertiary amino group are not always easy to synthesize. We have encountered such difficulties for example in the preparation of dimethylaminomethyloxirane (XI). Therefore, we examined the Mitsunobu reaction which generates the oxirane *in situ* from the commercially available 3-dimethylaminopropane-1,2-diol by treatment with ethyl azodicarboxylate and triphenylphosphine. The oxirane XI was directly used for alkylation of the heterocyclic base. The resulting 3-dimethylamino-2-hydroxypropyl derivatives XII were easily purified and converted to phosphonates If by the above standard procedures. The usefulness of this method has been documented by the synthesis of adenine and cytosine derivatives If (Scheme2).

The preparation of quaternary trimethylammoniummethyl derivatives Ih followed the obvious route which made use of the commercially available trimethylammoniummethyloxirane salt XIII. The alkylation of heterocyclic bases proceeded smoothly in the presence of a basic catalyst (disregarding the ionic character of the reagent); however, the high polarity of the condensation products XIV did not allow the separation of regioisomers either by ion exchange chromatography or by preparative HPLC. Therefore, the crude products were further transformed to the amidine-protected intermediates XV and without purification condensed with the synthon IV. After deprotection, the final quaternary phosphonates were isolated and purified by ion-exchange chromatography. This procedure yielded pure N^9-regioisomers Ih in both cases tested (adenine, 2,6-diaminopurine) (Scheme 2).

The above methods were used for the preparation of racemic phosphonomethoxyalkyl derivatives. We have also developed a method permitting to prepare pure enantiomers of aminomethyl derivatives Id by an enantiospecific synthesis: 3-O-trityl-(S)-oxirane (XVI) was first converted to (R)-azidopropanediol derivative XVII. Its condensation with p-toluenesulfonyloxymethylphosphonate IV in THF gave diester XVIII. Ion exchanger-catalyzed detritylation followed by tosylation of the intermediate XIX led to the final product XX. The use of this synthon was demonstrated by the preparation of guanine derivative XXIII. Condensation of 2-amino-6-chloropurine (sodium salt) with the synthon

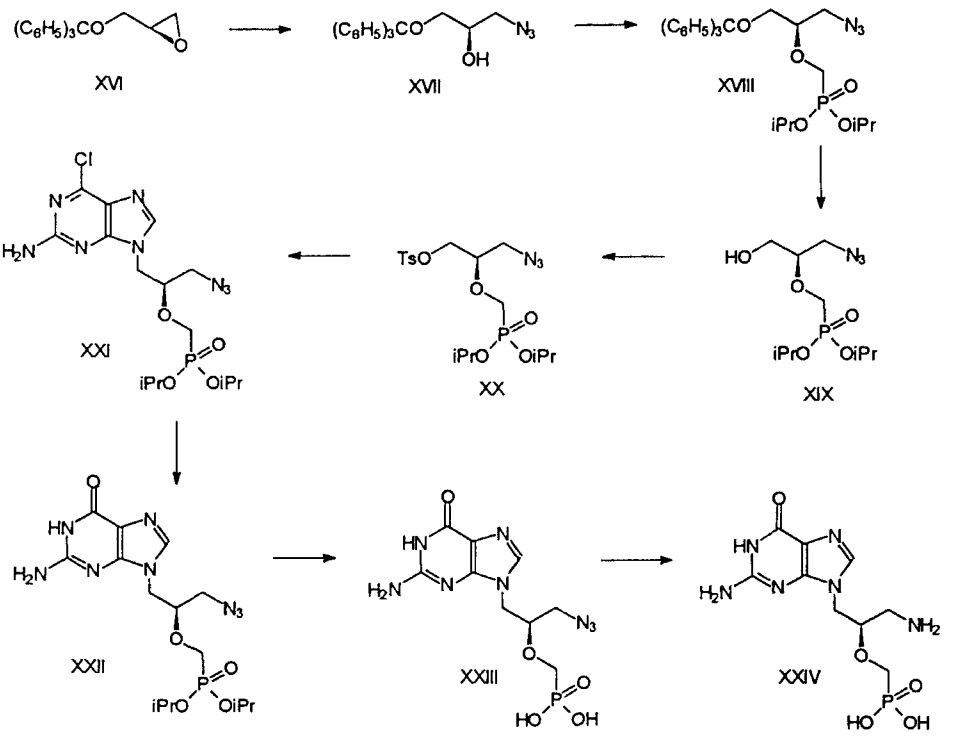


Scheme 2

XX and subsequent hydrolysis of diester XXI in the presence of 1,4-diazabicyclo-[2.2.2]-octane (DABCO)[18] gave guanine derivative XXII.Reaction with BTMS and hydrolysis afforded azido phosphonate XXIII which was then transformed to the (S)-enantiomer of compound XXIV by palladium-catalyzed hydrogenation (Scheme 3).

The resulting amino-substituted phosphonates were isolated as the free acid (zwitter-ionic) forms by ion exchange chromatography. Their physico-chemical data (NMR, MS) were compatible with the proposed structures. The basic character of the amino group manifests itself by markedly decreased electrophoretic mobility at slightly alkaline pH. As expected, the quaternary derivatives Ih show an extremely low mobility in the electric field under the experimental conditions.

Synthesis of base-modified HPMP-derivatives. Synthetic procedures for the preparation of acyclic nucleotide analogues are undergoing continuous development. There are two principal approaches, each of a specific utility: Introduction of the phosphonomethyl ether group into a preformed N-hydroxyalkyl derivative of the heterocyclic base is a method of choice for large-scale preparations[18,19]. It is also advantageous in the synthe-



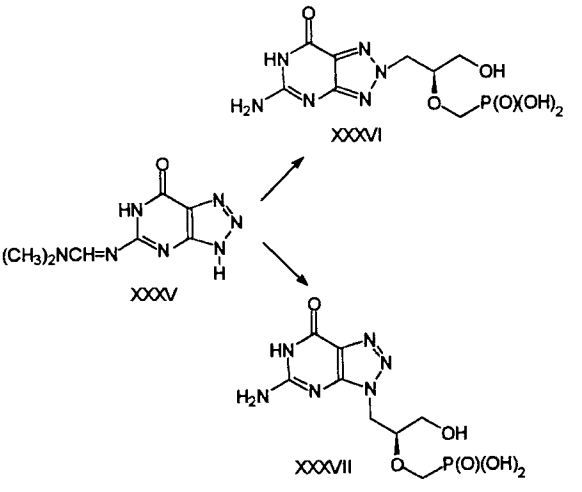
Scheme 3

ses of side chain-modified analogues[20]. It requires isolation and purification of the alkyl derivative that is usually obtained by alkylation of the heterocyclic base and, if needed, further specific protection at the base residue before the condensation proper. This procedure is not suitable for work in small quantities or in cases where the regiospecificity of the primary alkylation is low. In such situations, it is preferable to use other alternatives that employ synthons possessing all features of the future phosphonate-containing side-chain. The alkylation of the base is then the ultimate step of the synthesis and problems of availability of the base (reaction scale) or complexity of the regioisomeric mixture are thus minimal. In some cases (e.g.HPMP- or PMP-derivatives) the preparation of such synthons is complicated by chirality of the chain that requires enantiospecific control of the sequence. The applicability of the procedure is determined by the deprotection conditions. In the first case of HPMP-synthon, the protecting group (O-benzyl) had to be re-

moved by hydrogenolysis, a procedure unacceptable for some of the bases[21]. We have tried to circumvent this problem by using the pivaloyl group instead[22]. However, its use excludes the alkali-catalyzed introduction of phoshonomethyl ether residue rendering thus the method rather limited in scale.

We now report another preparation of both the (R)- and (S)-HPMP-synthon which starts from a common intermediate and which can be easily performed on a larger scale (Scheme 4). The key intermediate, 1-O-benzyl-(R)-glycerol (XXV), is easily available from 1,2:5,6-diisopropylidene-D-mannitol via 1,2-O-isopropylidene-(S)-glycerol. Compound XXV was tritylated and the product XXVI condensed in dry THF with diisopropyl

The utility of these synthons has been documented by condensation with adenine in the presence of cesium carbonate: the (R)-synthon XXXI gave, after methanolysis of the condensation mixture, (S)-HPMPA diisopropyl ester identical with the authentic material. This method was applied to the alkylation of 8-azaguanine: the base was first converted to the N^2-dimethylaminomethylene derivative XXXV by treatment with dimethylformamide dineopentyl acetal. Condensation with the synthon XXXI in the presence of cesium carbonate in DMF followed by alkaline hydrolysis with dilute ammonia produced a mixture of N^8- and N^9-regioisomers XXXVI, XXXVII (with predominating N^8-isomer)which were separated on silica. Removal of the diester groups by treatment with BTMS and subsequent hydrolysis yielded the isomers of 8-azaHPMPG (XXXVIII, XXXIX).



The antiviral activity of the above compounds was investigated by the virology group of the Rega Institute, Catholic University, Leuven (Head, Prof. E. De Clercq) by Drs. J. Balzarini and R.Snoeck. The results will be reported elsewhere.

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